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Shaping the chromatic characteristics of red wines by using biofilm-detached cells of *Starmerella bacillaris* strains

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Abstract

The aim of this study was to evaluate the effects of 10 *Starmerella bacillaris* strains inoculated as planktonic or biofilm-detached cells on the chromatic characteristics of Montepulciano d'Abruzzo wine. Wines inoculated with biofilm-detached cells of *St. bacillaris* were characterized by a higher content of glycerol and viable yeast cells and a lower content of ethanol than those obtained with planktonic cells. Pyruvic acid content ranged from 45.99 mg/L to 48.19 mg/L and from 41.13 mg/L to 45.9 mg/L in wines fermented with biofilm-detached and planktonic cells, respectively. Wines obtained with biofilm-detached cells showed levels of anthocyanins ranging from 506.8 mg/L to 659.9 mg/L, while those fermented with free cells of *St. bacillaris* ranged from 518 mg/L to 612.6 mg/L. Similarly, the content of polyphenols was higher in wines inoculated with biofilm-detached cells. The different amounts of these compounds resulted in differences in the wine's color. Wines obtained with biofilm-detached cells of *St. bacillaris* had lower b* and h* values than those obtained with planktonic cells. These wines also showed higher a* values, indicating the presence of a stronger red color than the others, and lower clarity (L*). Moreover, the data obtained highlighted that it is possible to predict the color of young wines from must measurements. Further studies will be done to evaluate the role of other non-Saccharomyces yeasts, grown under different aggregation states, in the definition of wine color.

Keywords: *Starmerella bacillaris*; biofilm-detached cells; planktonic cells; anthocyanins; wine color; Montepulciano d'Abruzzo

1. Introduction

The process of wine production encompasses a broad range of microorganisms with distinct functions (Jolly, Varela, & Pretorius, 2014). Yeasts and lactic acid bacteria (LAB) are the main components of the wine microbial consortium, and are known to exhibit either positive or negative effects (Jolly et al., 2014). Although *Saccharomyces cerevisiae* is commonly the dominant species, it is widely acknowledged that a diverse range of non-*Saccharomyces* yeasts are also present in both spontaneous and inoculated wine fermentations. Non-*Saccharomyces* yeasts play a significant role in the release of secondary metabolites contributing to the development of wine's flavor profile (Padilla, Gil, & Manzanares, 2016). In fact, these yeasts are involved in the production of esters, higher alcohols, acids and terpenes (for a review see Padilla et al., 2016). These compounds are essential in the definition of wine organoleptic properties and play an important role in consumer preference (Madžgalj et al., 2022).

During the fermentation process a metabolic interplay between *S. cerevisiae* and non-*Saccharomyces* yeast species has been described, indicating that they do not merely coexist in a passive manner (Jolly et al., 2014). For instance, mixed fermentation of *S. cerevisiae* and *Starmerella bacillaris* (also known as *Candida zemplinina*) allow to enhance fermentation kinetics while minimizing the production of ethyl acetate and acetic acid (Tofalo et al., 2016). This non-*Saccharomyces* yeast is commonly isolated from grapes, musts, soil, fruits, and insects, and exhibits noteworthy oenological traits e.g., elevated glycerol production, reduction of acetic acid and ethanol concentration, enhanced aroma complexity, capacity to thrive in high sugar concentrations, and fructophilic tendencies (Tofalo et al., 2012; Russo et al., 2020; Nadai, Giacomini, & Corich, 2021; Nisiotou et al., 2018). Moreover, the inoculation of *St. bacillaris* adhered on oak chips allowed to improve the color of Trebbiano Abruzzese wines improving its green/yellow nuances (Perpetuini et al., 2023). The impact of yeasts on wine color can be attributed to three distinct mechanisms. Firstly, yeasts can release metabolites that can contribute to the stabilization of red wine color and enhance the content of stable pigments (Escott, Feuillat, Dulau, & Charpentier, 2018). Secondly, yeasts possess enzymatic activities such as glycosidase and pectinase which favor polyphenols extraction form grapes. Finally, yeast cell walls have the ability to adsorb phenolic compounds, particularly anthocyanins and tannins, resulting in a significant reduction in red wine color and astringency (Tofalo, Suzzi, & Perpetuini, 2021). This phenomenon is strain dependent and not yet completely understood. It probably depends on cell wall surface structure and composition being apolar anthocyanins better adsorbed than polar ones. Moreover, the impact of yeasts on wine color is also related to the production of acetaldehyde and pyruvic acid (Morata et al., 2012; Belda et al., 2017). This activity is strain dependent and is notably pronounced in non-*Saccharomyces* yeasts. It has been observed that *Schizosaccharomyces pombe* released a greater concentration of pyruvate compared to *Saccharomyces cerevisiae*. In comparison to *S. cerevisiae*, *Torulaspora delbrueckii* is known to generate a reduced quantity of acetaldehyde. Therefore, the identification of non-*Saccharomyces* yeast strains exhibiting optimal pyruvate and acetaldehyde production for co-fermentation with *S. cerevisiae* could serve as a viable approach to stabilize wine color (Morata et al., 2012; Belda et al., 2017). It seems that the augmentation of metabolic activity and survival time of non-*Saccharomyces* yeasts can lead to a successful mixed culture fermentation also in terms of wine color.

Recently, there has been a growing interest in the biofilm lifestyle of yeast and bacteria. Biofilms can be defined as a community of microorganisms that are enclosed by an extracellular matrix composed of extracellular polymeric substances that are generated by the microorganisms themselves (Donlan & Costerton, 2002). Recent studies have reported that biofilm-detached cells are characterized by phenotypes and properties similar to sessile cells and different from those of planktonic ones (Perpetuini et al., 2021, Perpetuini, Tittarelli, Perla, & Tofalo, 2022; Bastard et al., 2016). Therefore, some authors suggested the use of sessile cells, as well as biofilm-detached cells, to shape the oenological parameters of red and white wines (Perpetuini et al., 2021; 2023; Bastard et al., 2016; Pannella et al., 2020). However, to date, little information is available on the influence of biofilm detached yeasts on the chromatic characteristics of wine. Therefore, the aim of this study was to evaluate the impact of biofilm-detached and planktonic cells of *St. bacillaris* in co-colture with *S. cerevisiae* on the chromatic characteristics of Montepulciano d'Abruzzo wine.

2. Materials and methods

2.1 Sampling site

Must samples *Vitis vinifera* cultivar (cv.) Montepulciano were kindly provided by a cellar located in Orsogna (Chieti, Abruzzo, Italy). Vineyards (42◦13′ 01.5′′N; 14◦14′ 43.6′′E), 403 m elevation, with calcareous, clayey soil received no irrigation, and were subjected to organic in accordance with Reg. EC 834/2007 (EC, 2007) since 2012. In particular, the pest management was achieved only through copper/sulphur-based products.

The *Vitis vinifera* cultivar (cv.) Montepulciano is the most important red variety of Abruzzo region, with over 35,000 ha of vineyards planted mainly along the Adriatic Coast. It is used for the production of high-quality red wines like Montepulciano d'Abruzzo Colline Teramane, and Terre Tollesi (or Tullum) wines which gained the DOCG (Designation of Controlled and Guaranteed Origin) recognition.

2.2 Strains origin

Ten strains of *St. bacillaris* (SB1, SB3, SB5, SB7, SB8, SB9, SB10, FUC9, FUC16, and FUC17) and a strain of *S. cerevisiae* (SRS1) were used in this study. All strains belong to the Culture Collection of the Microbial Biotechnology Laboratory (Department of BioScience and Technology for Food, Agriculture, and Environment – University of Teramo, Italy) and were previously characterized (Suzzi et al., 2012; Tofalo et al., 2016; Perpetuini et al., 2021). All strains were isolated from Montepulciano d'Abruzzo grapes, with the only exception of FUC9, FUC16, and FUC17 which were isolated from Nero Antico di Pretalucente grapes. The strains were cultivated under aerobic conditions at 28°C for 48 hours on YPD medium, which consists of 1% w/v yeast extract, 2% w/v peptone, and 2% w/v glucose, as per standard practice. The strains were preserved at a temperature of -80°C in YPD broth supplemented with glycerol (Sigma-Aldrich, Milan, Italy) at a final concentration of 20% v/v.

2.3 Small scale vinification

Must from Montepulciano grapes was obtained after 3 days of cryomaceration at 4 °C in contact with skins and seeds, was divided in aliquots of 400 mL, and pasteurized (Caridi, Sidari, Kraková, Kuchta, & Pangallo, 2015). Fermentations were carried out in 500 mL Erlenmeyer flasks closed with a Müller valve filled with sulfuric acid. Each flask contained 400 mL of the must obtained as described above (248 g/L – 24.6 °Bx of fermentable sugars, 7.67 titratable acidity, pH of 3.4). The fermentation was carried out under static conditions at 25°C. The flasks were inoculated with pre-cultures grown in the same must for 48 h. Strains were co-inoculated at a final concentration of 6 Log CFU/mL. The cell concentration was determined by counting under light microscopy. *Starmerella bacillaris* strains were inoculated both as planktonic and biofilm-detached cells. Biofilm-detached cells were prepared as previously described (Perpetuini et al., 2022). Briefly, biofilms were formed inoculating cells in flat-bottom 6-well cell culture plates (Costar, Corning, NY, USA). After 7 days sessile cells were detached using a sterile cell scraper (Perpetuini et al., 2022). These cells are referred as biofilm detached cells and used for further experiments.

The kinetics of fermentation were assessed on a daily basis through the observation of weight reduction resulting from the emission of CO2. Once a stable weight was reached, the fermentation process was considered as ended. Three biological and three technical replicates were conducted.

2.4 Viable yeasts count

Serial dilutions were prepared in physiological solutions (NaCl 0.85% w/v). Cell suspensions were plated on WLN agar, which allows the visual differentiation of *St. bacillaris* and *S. cerevisiae* yeast species. Plates were incubated at 28 °C for 3–5 days before counting. In this medium, *St. bacillaris* forms flat, light to intense green colonies, while *S. cerevisiae* forms creamy white colonies, with light shades of green on the top facilitating the concurrent enumeration of both species during the fermentation process. Plate count was performed after 7 days of alcoholic fermentation (T7) and at the end of fermentation (Tf). All analyses were performed in triplicate.

2.5 Main oenological parameters

FOSS WineScan™ FT120 rapid scanning Fourier Transform Infrared Spectroscopy with FOSS WineScan software version 2.2.1 was used to analyse the main physicochemical parameters. Previously, the equipment was calibrated using wine samples tested according to established OIV protocols (OIV, 2023). The pH was determined using a pH meter. Pyruvate, polyphenols, and anthocyanins were determined enzymatically using commercial kits from Steroglass (Perugia, Italy) according to the manufacturer's instructions. Acetaldehyde concentration was determined by gas chromatography with a flame ionization detector (GC-FID) using Agilent Technologies 6850 equipment (Palo Alto, CA), according to Morata et al. (2015).

2.6 Wine color analysis

Wine color analysis was carried out using a colorimeter (Minolta, Chroma Meter CR-5). Clarity (L*), red/green color component (a*), and blue/yellow color component (b*), and their derived magnitudes, chroma (C*), and tone (h*), were determined using glass cuvettes with a path length of 0.2 cm after clarification of the samples by centrifugation (OIV, 2023). The color of wines obtained with planktonic cells and biofilm-detached cells was compared, and the color difference was expressed as $\Delta E = [(\Delta L)2 + (\Delta a^*)2 + (\Delta b^*)2]$ ½ (Ayala, Echavarri, & Negueruela, 1997).

2.7 Confocal laser scanning microscopy

Biofilms were visualized through the utilization of confocal laser scanning microscopy (CLSM) with the Nikon A1-R confocal imaging system, which was operated through the Nikon NIS-Elements interface (Version 4.40, Nikon Corp., Tokyo, Japan). The analyses were conducted in triplicate

2.8 Statistical analysis

The ANOVA test was performed using XLStat 2014 software (Addinsoft, New York, NY, USA) and was applied on the oenological parameters, the content of polyphenols, anthocyanins, pyruvic acid, and acetaldehyde, and the chromatic characteristics of wine in order to identify the significant differences. The Bonferroni correction was applied. The Pearson's Correlation matrix analysis was performed using XLStat 2014 software considering the content of anthocyanins, polyphenols, glycerol, ethanol, pyruvic acid, acetaldehyde, the number of cells and the chromatic charactristics of wines.

Moreover, a machine learning (ML) framework to estimate the wine color from anthocyanins, polyphenols, the number of viable yeast cells, pyruvic acid, and acetaldehyde was developed. Particularly, a Support Vector Regressor (SVR) with a radial basis function kernel was used to estimate, independently, the L*, a*, and B features, using as input anthocyanins, polyphenols, the number of viable yeast cells, pyruvic acid, and acetaldehyde. The input features were normalized (z score), and the generalization performance of the model was tested employing a nested cross validation (nCV). The nCV approach involves partitioning the available data into distinct folds, and subsequently training the model in an iterative and nested manner on all folds except for one. The outer loop and inner loop serve distinct purposes in the model evaluation process. While the outer loop is responsible for estimating the model's performance across iterations, the inner loop is tasked with identifying the optimal hyperparameter through validation. In this study, a 5-fold CV was performed. The performance of the models was evaluated considering the correlation coefficient between the measured and predicted variables.

3. Results and discussion

The microbial metabolism is influenced by the lifestyle of microrganisms: sessile cells, as well as biofilm-detached cells, frequently express phenotypes that are different from their planktonic counterparts (Bastard et al., 2016; Pannella et al., 2020). Recent studies reported the ability of *St.*

bacillaris to form biofilms on different abiotic surfaces, revealing that sessile and planktonic cells can influence the characteristics of wines in different ways (Perpetuini et al., 2021; 2022). In particular, wines fermented with sessile cells allowed to obtain wines with higher concentrations of esters and glycerol and with a different sensory profile. In order to better understand the contribution of biofilm-detached cells to wine characteristics, in this study, the effect of biofilm-detached cells and planktonic cells on the chromatic characteristics of Montepulciano d'Abruzzo wine was tested.

3.1 Determination of biofilm forming ability

The biofilms formed by *St. bacillaris* strains were visualized, for the first time, by CSLM. CSLM analysis revealed that all strains were able to form biofilm, in a strain-dependent way. Fig. 1 showed a three-dimensional reconstruction of *St. bacillaris* biofilms resulting from the compilation of a series of individual xy sections taken across the z axis. The images showed a biofilm organized in a monolayer of sessile cells surrounded by an extracellular polysaccharide-like substance. Although, the biofilm did not cover the entire surface of the glass, the cells adhered, flattened, and produced extracellular material that bonded them to the surface, after which they finally organized themselves in microcolonies (Fig. 1).

3.2 Oenological parameters and yeast viability

The presence of *S. cerevisiae* allowed the fermentation process to end after 15 days. However, when *St. bacillaris* was inoculated as biofilm-detached cells, a slower fermentative activity was observed (Supplementary Figure 1). In fact, the trials inoculated with *S. cerevisiae* and biofilm-detached cells of *St. bacillaris* showed a lower fermentative power, evaluated as CO_2 evolution (g/100 ml) after 2 days of fermentation. The CO_2 evolved in trials inoculated with *S. cerevisiae* and planktonic cells of *St. bacillaris* ranged from 1.6 g CO*2*/100mL to 5.1 *g* CO2/100mL, while in trials inoculated with *S. cerevisiae* and biofilm-detached cells of *St. bacillaris* from 0.88 g CO_2/100mL to 3.65 g CO_2/100 mL after 2 days. This slower fermentation ability could be related to the metabolism of sessile cells or biofilm-detached cells, which are characterized by a different metabolism, e.g., in terms of metabolite production, than their planktonic counterparts (Bojsen et al., 2012). Probably, these differences could slow down the fermentation process, influencing the interactions between *St. bacillaris* and *S. cerevisiae* strains. Rossouw et al. (2015, 2018) showed that changes in adhesion properties of *S. cerevisiae* significantly affected the survival of other yeast species. Probably, this evidence could be true also for *St. bacillaris* strains used in this study. Moreover, the inoculation of biofilm-detached or planktonic cells of *St. bacillaris* could cause a differential expression of S. cerevisiae genes involved in the fermentation process (Pourcelot et al., 2023). It should be also noted that, the different yeast species could have overlapping nutritional requirements leading to competition for nutrients such as amino-acids or vitamins (Evers et al. 2021). Probably, biofilm-detached cells could be more competitive with *S. cerevisiae* and steal nutrients from it during the first steps of alcoholic fermentation. This observation is in agreement with previous studies which highlighted that different couples of *St. bacillaris* and *S. cerevisiae* can influence the growth dynamics, the fermentation behavior and, as a consequence, wine composition in a couple-dependent manner (Englezos et al., 2019). On the basis of our results, the interaction between these 2 yeasts is not only couple-ependent, but depends also on *St. bacillaris* lifestyle.

The lifestyle of *St. bacillaris* did not influence the main oenological parameters of Montepulciano d'Abruzzo wines (Table 1). Significant differences were only observed for the content of ethanol and glycerol. A slight reduction of ethanol was detected when *St. bacillaris* was inoculated as biofilm detached cells. Probably, in biofilm-detached cells the acetaldehyde pathway is less active than in planktonic ones. In fact, when biofilm-detached cells are inoculated a reduction of ethanol content and an increase of glycerol concentration have been detected. Effectively, the low production of ethanol is strictly linked to the low activity of the acetaldehyde pathway. It is already known that this behaviour has large-scale effects on the metabolic fluxes, necessitating higher glycerol production to compensate for reduced ethanol production and to maintain cells' redox balance (Ansell, Granath, Hohmann, Thevelein, & Adler, 1997) (Fig. 2). As a direct consequence, increased production of pyruvate and amino acids and larger amounts of alcohols derived from alanine, leucine, valine, and isobutanol, as well as metabolites from glyceraldehyde-3-phosphate, are shown (Comitini et al., 2021).

Glycerol is the most abundant yeast metabolism by-product after ethanol and CO_2. This is a non volatile 3-hydroxy alcohol and appears to contribute to the mouthfeel and sweetness of wine in the range of 5–12 g/L (Ivit, Longo, & Kemp, 2020). Wines obtained with biofilm-detached cells of *St. bacillaris* were characterized by a higher content of glycerol than those obtained with planktonic cells. In particular, wines produced with biofilm-detached cells produced wines with a content of glycerol ranging from 6.06 g/L (SRS1+SB8) to 9.38 g/L (SRS1+SB9), while the planktonic ones ranged from 5.03 g/L (SRS1+SB7) to 8.12 g/L (SRS1+FUC17) (Table 1). Similar results have already been reported when *St. bacillaris* was adhered to oak chips (Perpetuini et al., 2021; 2023). The glycerol biosynthetic genes are up-regulated in biofilms, and the amounts of glycerol are significantly higher in sessile cells compared to planktonic cells (Desai et al., 2013). In fact, the decreased glycerol levels result in the down-regulation of biofilm adhesin genes such as ALS1, ALS3, and HWP1 (Desai et al., 2013). It is unclear why glycerol and biofilm formation should be so closely linked. However, according to Desai et al. (2013) glycerol biosynthesis is essential for proper expression of numerous biofilm regulated genes, including adhesin genes. The obtained results highlighted that the number of *St. bacillaris* viable yeast cells in the fermentation trials performed with planktonic cells was characterized by a stronger cell decay ($p < 0.05$) than that observed in trials fermented with biofilm detached ones after 7 days of fermentation. In fact, a decrease of about 2 Log CFU/mL was observed: the number of *St. bacillaris* viable cells in fermentation trials performed with planktonic cells showed a mean value of about 2.42 log CFU/mL, while that of trials inoculated with biofilm-detached cells was 3.91 log CFU/mL (Fig. 3). On the contrary, the number of *S. cerevisiae* viable cells was similar in both conditions. At the end of alcoholic fermentation, the number of biofilm-detached *St. bacillaris* cells was about 2 Log CFU/mL, while this yeast was not detected in the trials performed with planktonic cells. *S. cerevisiae* cells were detected in similar concentration (Fig. 3). This finding could be related to the ability of biofilm-detached cells to better face the stresses of alcoholic fermentation. In fact, as reported by Guilhen et al. (2016), cells dispersed from biofilms have a high stress response because they are transcriptionally closer to their parent cells in biofilm form than to cells in planktonic form.

3.3 Biofilm-detached cells increase the content of pyruvic acid, anthocyanin, and polyphenols

Acetaldehyde is a potent volatile flavor compound that, at low levels, gives a pleasant fruity aroma, but at high concentrations (higher than 100–125 mg/L), it possesses a pungent, irritating odor (Berg, Filipello, Hinreiner, & Webb, 1955). Moreover, it plays a key role in the increase in color (Liu & Pilone, 2000). However, it should be noted that the International Agency for Research on Cancer (IARC) classified acetaldehyde as “possibly carcinogenic to humans (Group 2B)” and, in combination with its oral intake via alcoholic beverages, as “carcinogenic to humans (Group 1)”. According to the criteria set out in Regulation (EC) No 1272/2008 (Classification, Labeling and Packaging regulation), acetaldehyde is classified as carcinogenicity category 1B (may cause cancer) and germ cell mutagenicity category 2 (suspected of causing genetic defects) meeting the criteria to be considered a carcinogenic, mutagenic, and/or toxic for reproduction (Cartus et al., 2023).

Acetaldehyde content was similar in both conditions; in fact, a mean value of 40 mg/L was detected in wines obtained with planktonic and biofilm-detached cells.

The content of pyruvic acid was higher in wines obtained with biofilm-detached cells. In particular, its content ranged from 45.99 mg/L (SRS1+SB10) to 48.19 mg/L (SRS1+FUC17) and from 41.13 mg/L (SRS1+SB9) to 45.9 mg/L (SRS1+FUC16) in wines fermented with biofilm-detached and planktonic cells, respectively (Table 2). It seems that biofilm-detached cells are more efficient at redirecting sugar consumption for the production of alternative compounds, rather than ethanol, than planktonic ones. These alternative compounds could be glycerol and pyruvic acid produced via glycerol-pyruvic metabolisms (Fig. 2). The production of pyruvic acid has already been described in *St. bacillaris* (Magyar, Nyitrai-Sárdy, Leskó, Pomázi, & Kállay, 2014; Mangani, Buscioni, Collina

Bocci, & Vincenzini, 2011). Generally, the production of pyruvate by wine yeasts varies from 50 mg/L to 120 mg/L (Morata, Gómez-Cordovés, Colomo, & Suárez, 2003). Generally, the production of pyruvate, a metabolic intermediate in the biosynthesis of acetyl CoA, grows at the beginning of fermentation, while its concentration decreases at the end of alcoholic fermentation. As the fermentation process progresses and the availability of nutrients decreases, yeasts utilize the pyruvate that was previously secreted during the earlier stages of fermentation (Morata et al., 2003).

The production of pyruvic acid is essential to improving wine color. In fact, according to Morata et al. (2003), a linear relationship between vitisin A production and pyruvate levels can be observed. Therefore, the use of biofilm-detached cells, characterized by a higher production of pyruvic acid than planktonic ones, could be an interesting strategy to modulate wine color. This may be especially important for red wines destined to be aged (especially if they are aged in the barrel) or are to undergo a second fermentation (e.g., sparkling wines). The color of wine is also influenced by anthocyanins and polyphenols, as well as the extraction, absorption and preservation phenomena of anthocyanins. Therefore, their content was also evaluated. The anthocyanins were mainly absorbed by planktonic cells; in fact, wines obtained with biofilm-detached cells showed levels of anthocyanins ranging from 506.8 mg/L (SRS1+FUC16) to 659.9 mg/L (SRS1+SB7), while those fermented with free cells of *St. bacillaris* ranged from 518.8 mg/L (SRS1+FUC9) to 612.6 mg/L (SRS1+SB1) (Table 2). Similarly, the content of polyphenols was higher in wines inoculated with biofilm-detached cells. In fact, the content of polyphenols ranged from 5.7 g/L gallic acid equivalents to 6.9 g/L gallic acid equivalents, and from 5 g/L gallic acid equivalents to 5.7 g/L gallic acid equivalents in biofilm-detached and planktonic cells, respectively.

The concentration of polyphenols in wines is influenced by viticulture (grape variety and clone, light exposure, degree of ripeness), yeast strains, and vinification process (destemming, crushing, pre fermentation maceration, alcoholic fermentation, pressing) (Jagatic Korenika, Tomaz, Preiner, Plichta, & Jeromel, 2021). For instance, according to Lisov et al. (2020) the extraction of phenolic compounds during alcoholic fermentation is affected by maceration time. The best results were obtained after 15 days of maceration, with exceptions of gallic acid, catechin, and myricetin. Regardless of the adhesion properties of *St. bacillaris*, a negative relationship can be established between the number of viable yeast and the content of anthocyanins and polyphenols, suggesting that their release or adsorption is mainly dependent on the vitality of yeasts. Probably, the differences observed in this study could be related to the viability of the yeast cells. In fact, cells embedded in a biofilm, as well as biofilm-detached cells, are more resistant to stresses than planktonic ones. According to Echeverrigaray, Scariot, Menegotto, and Delamare (2020), a negative correlation between pigment adsorption and both cell viability and cell wall/membrane integrity can be observed. Irrespective of their adsorptive potential during the process of wine fermentation, viable cells demonstrated a limited ability to adsorb anthocyanins. Conversely, permeabilized yeast cells exhibited a high capacity for pigment adsorption.

3.4 Oenological parameters and wine color

The color of red wine is a major concern for the wine industry since it strongly affects consumer demands. Anthocyanin content is the main reason for the color of red wine and depends on the grape variety, degree of grape ripeness, soil, and climatic conditions. It undergoes a progressive change from production to consumption of any wine due to polymerization, copigmentation, and oxidation reactions. Therefore, it is important to evaluate the effect of the different oenological parameters on wine color and try to predict it on the basis of these parameters.

Concerning the chromatic characteristics of wine, b* values (blue/yellow color) were all low, reflecting the low presence of yellow color component in Montepulciano d'Abruzzo wines (Table 3). Wines obtained with biofilm-detached cells of *St. bacillaris* had lower values of b* and h* than those obtained with planktonic cells. The lower value of h* leads to purple or ruby red, while higher values lead to brick red or reddish brown. These wines also showed higher a* values, indicating the presence of a stronger red color than the others, and lower clarity (L*) (Table 3). No significant differences were obtained for the parameter c*, which represents the psychometric chroma. It is important to underline that in 6 trials out of 10, the E values were higher than 3 CIELAB units (Table 4), indicating that the color differences between wines obtained from planktonic and biofilm-detached cells could be perceived by human eyes (Martinez, Melgosa, Perez, Hita, & Negueruela, 2001). These results suggested that yeast's absorption of phenolic compounds could result in an increase in yellow color and a reduction of blue and red nuances, indicating that not only the choice of yeast strains but also their lifestyle (planktonic vs. biofilm-detached) is important to defining the color of wine. The content of anthocyanins could help explain these differences. In fact, the content of anthocyanins is negatively correlated with L* values, suggesting their significant contribution to color intensity (i.e., a smaller L* value), and positively with a*, indicating their contribution to red wine color.

A correlation matrix was constructed to establish the relationship between the variables considered. In biofilm-detached cells, anthocyanin content was positively correlated with the concentration of polyphenols, the number of cells, and a* values. Positive relations were also present between polyphenols, a* values, and the number of cells. a* values were positively correlated with the number of cells and the content of acetaldehyde (Table 5). In planktonic cells, anthocyanins were positively correlated with the concentration of polyphenols and the number of cells. Polyphenols were positively related to the number of cells and a* values and negatively to pyruvic acid. a* values correlated positively with the number of cells and negatively with the amount of pyruvic acid. As expected, the content of polyphenols and anthocyanins is essential to improve red wine color in both conditions. Moreover, the number of viable cells is another key factor for the determination of red wine color. In fact, viable cells show a limited ability to adsorb anthocyanins on their cell wall (Echeverrigaray et al., 2020).

A regression model was developed to predict the color of wine based on the following parameters: anthocyanins, polyphenols, the number of viable yeasts, pyruvic acid, and acetaldehyde. The developed model behaved fairly well for the prediction of L*, a*, and b* when biofilm detached cells are inoculated. In fact, r2 was 0.727, 0.878, and 0.628 for L*, a*, and b*, respectively. Good regression coefficients were observed also for planktonic cells: r2 values of 0.628, 0.748, and 0.623 for L*, a*, and b*, respectively (Fig. 4). The regression coefficient of determination of cross-validation showed that the analysis of wine samples with these methods could allow predictions of wine color. It should be noted that the regression model behaved better in the presence of biofilm-detached cells, suggesting that their use in winemaking could be useful to predict the color of wine more accurately. However, to increase the accuracy and robustness of these prediction models and to employ them in commercial applications, larger sample sets can be used in future studies.

4. Conclusion

The results obtained in this study offer first evidence of the role of *St. bacillaris* grown as biofim detached cells in the determination of Montepulciano d'Abruzzo wine color. In particular, the co inoculation of biofilm-detached cells of *St. bacillaris* and *S. cerevisiae* resulted in an increase of glycerol, pyruvic acid, polyphenols and anthocyanins and a decrease of ethanol content. Moreover, wines obtained with biofilm-detached cells had lower values of b* and h* and higher a* values, indicating the presence of a stronger red color. Moreover, it should possible to predict the color of young wines from must measurements. The developed model behaved fairly well for the prediction of L*, a*, and b* when biofilm detached cells were inoculated. This approach provides an important starting point for further identification and prediction of wine quality factors from these parameters. This kind of studies are of great importance to help the oenologists to better manage wine polyphenols through the correct choice of yeast strain or inoculum strategy.

CRediT authorship contribution statement Rosanna Tofalo: conceptualization, supervision, funding acquisition, Writing – review & editing. Luca Valbonetti: CLSM analysis. Rossana Sidari: investigation. Alessio Pio Rossetti: investigation, formal analysis. Giorgia Perpetuini: formal analysis, data curation, Writing – original draft, Writing – review & editing. Carlo Perla: Writing – review & editing, Camillo Zulli: Writing – review & editing

Declaration of Competing Interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability Data will be made available on request.

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Figure captions

Figure 1. CLSM images of *St. bacillaris*. (A) ×100 3D images of strains. (B) ×100 3D images from the frontal view of strains.

Figure 2. Carbon metabolism in yeasts. ADH: alcohol dehydrogenase; GPDH: glycerol-3-phosphate dehydrogenase; G3P: glycerol-3-phophatase; PDC: pyruvate decarboxylase; DHAP: dihydroxyacetone phosphate; GA3P: glyceraldehyde-3-phosphate.

Figure 3. Box plot showing the number of viable yeasts after 7 days (T7) and at the end of alcoholic fermentation (Tf). PL: planktonic, BD: biofilm-detached. ns: $p>0.05$, *$p<0.05$

Figure 4. Correlation between obtained L*, a* and b* values and predicted ones.

Supplementary Figure 1. Fermentation kinetics. P: planktonic, BD: biofilm-detached

Table 1 Main oenological parameters obtained at the end of alcoholic fermentation using co-cultures of *S. cerevisiae* and *St. bacillaris* grown as planktonic or sessile cells. Different letters in the same line indicates significant differences ($p<0.05$)

Trial	Alcohol (% v/v)		Residual sugars (g/L)		pH		Titratable acidity (g/L)*		Volatile acidity (g/L)**		Glycerol	
	Biofilm-detached	Planktonic	Biofilm-detached	Planktonic	Biofilm-detached	Planktonic	Biofilm-detached	Planktonic	Biofilm-detached	Planktonic	Biofilm-detached	Planktonic
SRS1+SB1	13.92 ± 0.32^{A}	14.12 ± 0.32^{A}	0.57 ± 0.03^{A}	0.55 ± 0.03^{A}	3.33 ± 0.13^{A}	3.31 ± 0.05^{A}	5.39 ± 0.33^{A}	5.37 ± 0.43^{A}	0.52 ± 0.03^{A}	0.53 ± 0.08^{A}	7.54 ± 0.23^{B}	5.36 ± 0.44^{A}
SRS1+SB3	13.74 ± 0.83^{A}	14.24 ± 0.53^{A}	0.34 ± 0.08^{A}	0.36 ± 0.03^{A}	3.3 ± 0.27^{A}	3.32 ± 0.17^{A}	6.44 ± 0.37^{A}	6.43 ± 0.84^{A}	0.45 ± 0.03^{A}	0.48 ± 0.03^{A}	7.97 ± 0.44^{B}	5.27 ± 0.35^{A}
SRS1+SB5	14.25 ± 0.54^{A}	14.15 ± 0.99^{A}	0.36 ± 0.03^{A}	0.31 ± 0.02^{A}	3.33 ± 0.08^{A}	3.35 ± 0.14^{A}	6.29 ± 0.12^{A}	6.21 ± 0.32^{A}	0.45 ± 0.08^{A}	0.48 ± 0.04^{A}	8.89 ± 0.43^{B}	6.89 ± 0.93^{A}
SRS1+SB7	13.71 ± 0.78^{A}	13.93 ± 0.13^{A}	0.59 ± 0.04^{A}	0.51 ± 0.04^{A}	3.33 ± 0.15^{A}	3.34 ± 0.34^{A}	6.66 ± 0.93^{A}	6.73 ± 0.34^{A}	0.49 ± 0.07^{A}	0.51 ± 0.09^{A}	6.14 ± 0.22^{B}	5.03 ± 0.56^{A}
SRS1+SB8	13.73 ± 0.23^{A}	14.16 ± 0.23^{A}	0.33 ± 0.06^{A}	0.31 ± 0.07^{A}	3.32 ± 0.07^{A}	3.31 ± 0.14^{A}	6.67 ± 0.23^{A}	6.65 ± 0.98^{A}	0.48 ± 0.03^{A}	0.49 ± 0.02^{A}	6.06 ± 0.89^{B}	5.33 ± 0.29^{A}
SRS1+SB9	13.82 ± 0.67^{A}	14.18 ± 0.43^{A}	0.36 ± 0.06^{A}	0.31 ± 0.03^{A}	3.34 ± 0.16^{A}	3.33 ± 0.04^{A}	6.43 ± 0.32^{A}	6.3 ± 0.67^{A}	0.58 ± 0.02^{A}	0.57 ± 0.06^{A}	9.38 ± 0.77^{B}	8.1 ± 0.93^{A}
SRS1+SB10	13.77 ± 0.37^{A}	14.23 ± 0.57^{A}	0.24 ± 0.03^{A}	0.22 ± 0.02^{A}	3.31 ± 0.21^{A}	3.33 ± 0.17^{A}	6.7 ± 0.53^{A}	6.43 ± 0.75^{A}	0.56 ± 0.13^{A}	0.58 ± 0.11^{A}	9.16 ± 0.98^{B}	6.87 ± 0.37^{A}
SRS1+FUC9	13.81 ± 0.92^{A}	14.16 ± 0.65^{A}	0.31 ± 0.05^{A}	0.32 ± 0.04^{A}	3.3 ± 0.05^{A}	3.3 ± 0.07^{A}	6.12 ± 0.32^{A}	5.97 ± 0.09^{A}	0.41 ± 0.05^{A}	0.43 ± 0.07^{A}	9.08 ± 0.32^{B}	7.18 ± 0.36^{A}
SRS1+FUC16	13.92 ± 0.12^{A}	14.25 ± 0.22^{A}	0.33 ± 0.06^{A}	0.34 ± 0.03^{A}	3.29 ± 0.05^{A}	3.31 ± 0.08^{A}	5.61 ± 0.98^{A}	5.65 ± 0.73^{A}	0.46 ± 0.08^{A}	0.48 ± 0.03^{A}	9.13 ± 0.66^{B}	7.17 ± 0.32^{A}
SRS1+FUC17	13.51 ± 0.43^{A}	14.33 ± 0.84^{B}	0.39 ± 0.05^{A}	0.38 ± 0.02^{A}	3.28 ± 0.03^{A}	3.31 ± 0.13^{A}	5.78 ± 0.32^{A}	5.72 ± 0.12^{A}	0.45 ± 0.03^{A}	0.42 ± 0.05^{A}	9.19 ± 0.43^{B}	8.12 ± 0.76^{A}

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* Expressed as tartaric acid.

** Expressed as acetic acid.

Table 2 Anthocyanins, and polyphenols content at the end of alcoholic ferm entation using co-cultures of *S. cerevisiae* and *St. bacillaris* grown as planktonic or sessile cells. Different letters in the same line indicates significant differences ($p<0.05$)

Strain	Pyruvic acid (mg/L)		Anthocyanins (mg/L)		Polyphenols (g/L)		Acetaldehyde (mg/L)	
	Biofilm-detached	Planktonic	Biofilm-detached	Planktonic	Biofilm-detached	Planktonic	Biofilm-detached	Planktonic
SRS1+SB1	46.96±6.98^{B}	41.46±6.77^{A}	616.42±56.91^{A}	612.61±45.19^{A}	6.93±2.81^{B}	5.12±0.42^{A}	39.32±12.76^{A}	40.22±13.78^{B}
SRS1+SB3	47.23±12.54^{B}	42.63±5.92^{A}	604.83±43.13^{A}	599.53±67.31^{A}	6.34±0.62^{A}	5.75±0.33^{A}	32.13±9.54^{A}	32.59±9.54^{A}
SRS1+SB5	47.87±11.65^{B}	43.81±11.41^{A}	570.61±57.94^{B}	560.25±53.15^{A}	5.72±1.33^{A}	5.36±0.55^{A}	30.38±3.87^{A}	30.16±8.33^{A}
SRS1+SB7	47.09±9.54^{B}	41.77±13.76^{A}	659.95±28.15^{B}	539.76±65.93^{A}	6.84±0.41^{A}	5.22±1.37^{A}	43.77±12.77^{B}	45.34±10.65^{A}
SRS1+SB8	46.13±5.99^{B}	42.66±10.54^{A}	588.32±92.72^{B}	537.91±89.41^{A}	5.95±1.75^{A}	5.14±0.69^{A}	50.45±11.23^{A}	49.41±9.45^{A}
SRS1+SB9	47.55±15.61^{B}	41.13±6.98^{A}	632.74±72.56^{B}	523.94±36.12^{A}	6.76±1.26^{B}	5.26±0.83^{A}	38.67±6.45^{A}	38.56±14.34^{A}
SRS1+SB10	45.99±8.43^{B}	43.68±12.81^{A}	643.81±55.91^{B}	544.62±33.62^{A}	6.93±0.54^{B}	5.32±0.55^{A}	41.56±9.87^{A}	41.76±9.65^{A}
SRS1+FUC9	46.08±7.23^{B}	44.80±13.86^{A}	593.74±69.23^{B}	518.14±98.13^{A}	5.95±1.27^{A}	5.15±1.13^{A}	37.98±12.65^{A}	39.54±7.33^{B}
SRS1+FUC16	47.54±5.72^{B}	45.91±9.66^{A}	506.86±73.85^{A}	583.86±88.35^{B}	5.72±0.93^{A}	5.23±0.55^{A}	41.33±8.65^{A}	41.98±18.45^{A}
SRS1+FUC17	48.19±13.66^{B}	44.43±4.88^{A}	581.11±95.43^{A}	608.17±93.43^{B}	5.86±1.55^{A}	5.22±1.12^{A}	45.65±13.77^{B}	44.12±13.66^{A}

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Table 3 Main chromatic characteristics of obtained wines. Different letters in the same line indicates significant differences ($p<0.05$)

Trial	L*		a*		b*		C*		h*	
	Biofilm-detached	Planktonic	Biofilm-detached	Planktonic	Biofilm-detached	Planktonic	Biofilm-detached	Planktonic	Biofilm-detached	Planktonic
SRS1+SB1	34.51 ± 12.78^{A}	36.92 ± 9.54^{A}	44.74 ± 9.54^{B}	43.01 ± 9.32^{A}	5.21 ± 0.34^{A}	6.27 ± 0.53^{A}	43.58 ± 16.88^{A}	43.01 ± 16.75^{A}	6.46 ± 0.76^{A}	6.64 ± 0.56^{A}
SRS1+SB3	36.84 ± 15.43^{A}	36.97 ± 12.43^{A}	43.45 ± 11.65^{A}	43.13 ± 16.98^{A}	5.38 ± 0.53^{A}	5.8 ± 1.09^{A}	43.65 ± 9.43^{A}	43.35 ± 14.85^{A}	6.31 ± 1.16^{A}	6.42 ± 1.27^{A}
SRS1+SB5	34.08 ± 12.99^{A}	37.21 ± 6.54^{B}	42.78 ± 16.87^{A}	42.75 ± 12.54^{A}	5.91 ± 1.12^{A}	5.99 ± 0.37^{A}	43.68 ± 14.67^{A}	43.5 ± 12.37^{A}	6.48 ± 0.59^{A}	6.59 ± 0.54^{A}
SRS1+SB7	34.34 ± 13.87^{A}	37.15 ± 19.54^{B}	44.66 ± 12.34^{B}	42.78 ± 19.22^{A}	5.71 ± 0.76^{A}	5.85 ± 1.45^{A}	42.88 ± 16.43^{B}	41.97 ± 11.84^{A}	6.51 ± 0.27^{A}	6.98 ± 1.24^{A}
SRS1+SB8	35.61 ± 17.54^{A}	37.99 ± 12.66^{A}	43.97 ± 16.23^{B}	42.03 ± 16.16^{A}	5.53 ± 1.11^{A}	5.68 ± 0.65^{A}	42.71 ± 13.99^{A}	42.27 ± 9.86^{A}	6.15 ± 0.39^{A}	6.29 ± 1.18^{A}
SRS1+SB9	36.87 ± 11.43^{A}	37.11 ± 13.18^{A}	43.5 ± 17.93^{A}	42.84 ± 8.44^{A}	5.2 ± 0.85^{A}	5.92 ± 1.12^{A}	43.12 ± 11.59^{A}	42.71 ± 11.43^{A}	5.54 ± 0.51^{A}	6.67 ± 0.54^{B}
SRS1+SB10	35.22 ± 16.88^{A}	37.22 ± 15.32^{B}	43.66 ± 12.66^{B}	42.29 ± 12.66^{A}	5.86 ± 1.77^{A}	5.96 ± 0.87^{A}	43.95 ± 16.32^{A}	43.45 ± 16.58^{A}	6.53 ± 1.06^{A}	6.75 ± 1.49^{A}
SRS1+FUC9	34.78 ± 13.75^{A}	37.71 ± 12.98^{B}	42.91 ± 11.28^{B}	42.11 ± 16.92^{A}	5.49 ± 0.34^{A}	5.81 ± 1.66^{A}	43.52 ± 7.48^{A}	43.14 ± 9.38^{A}	5.97 ± 0.48^{A}	6.35 ± 0.75^{A}
SRS1+FUC16	37.09 ± 12.99^{A}	37.34 ± 14.31^{A}	42.67 ± 9.99^{B}	42.22 ± 16.32^{A}	5.54 ± 1.49^{A}	5.35 ± 0.59^{A}	42.51 ± 8.59^{A}	42.41 ± 14.44^{A}	5.81 ± 1.15^{A}	6.14 ± 0.98^{A}
SRS1+FUC17	35.31 ± 12.43^{A}	36.98 ± 17.77^{B}	43.98 ± 16.43^{B}	42.18 ± 11.05^{A}	5.56 ± 0.55^{A}	5.49 ± 1.15^{A}	43.99 ± 12.49^{B}	42.98 ± 17.51^{A}	5.88 ± 0.59^{A}	6.37 ± 1.16^{B}

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Table 4 Colour difference in CIELAB units (ΔE) between the wines derived from the inoculation of *S. cerevisiae* and *St. bacillaris* grown as planktonic and biofilm-detached cells

Trial	ΔE
SRS1+SB1	3.15
SRS1+SB3	0.54
SRS1+SB5	3.13
SRS1+SB7	3.38
SRS1+SB8	3.07
SRS1+SB9	1
SRS1+SB10	2.43
SRS1+FUC9	3.05
SRS1+FUC16	0.57
SRS1+FUC17	4.09

Table 5. Correlation matrix for samples obtained with biofilm-detached (A) and planktonic (B) cells

A

	Anthocyanins	Polyphenols	Glycerol	Ethanol	Cells	Pyruvic acid	Acetaldehyde	L*	a*	b*
Anthocyanins	1	0.820	-0.307	-0.324	0.903	-0.353	0.058	-0.163	0.644	0.070
Polyphenols	0.820	1	-0.460	-0.197	0.832	-0.228	0.142	0.119	0.781	0.117
Glycerol	-0.307	-0.460	1	0.169	-0.314	0.336	-0.441	-0.368	-0.647	-0.148
Ethanol	-0.324	-0.197	0.169	1	-0.469	0.162	-0.646	-0.087	-0.456	0.396
Cells	0.903	0.832	-0.314	-0.469	1	-0.476	0.094	0.045	0.624	-0.009
Pyruvic acid	-0.353	-0.228	0.336	0.162	-0.476	1	-0.295	-0.277	-0.119	-0.132
Acetaldehyde	0.058	0.142	-0.441	-0.646	0.094	-0.295	1	0.215	0.483	-0.091
L*	-0.163	0.119	-0.368	-0.087	0.045	-0.277	0.215	1	0.014	-0.614
a*	0.644	0.781	-0.647	-0.456	0.624	-0.119	0.483	0.014	1	0.208
b*	0.070	0.117	-0.148	0.396	-0.009	-0.132	-0.091	-0.614	0.208	1

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B

	Anthocyanins	Polyphenols	Glycerol	Ethanol	Cells	Pyruvic acid	Acetaldehyde	L*	a*	b*
Anthocyanins	1	0.679	-0.102	0.523	0.726	0.102	-0.180	0.203	0.441	-0.297
Polyphenols	0.679	1	-0.476	0.079	0.701	-0.503	-0.341	-0.210	0.657	0.435
Glycerol	-0.102	-0.476	1	0.269	-0.232	0.389	-0.058	0.504	-0.348	-0.500
Ethanol	0.523	0.079	0.269	1	0.035	0.528	-0.131	0.523	-0.175	-0.492
Cells	0.726	0.701	-0.232	0.035	1	-0.215	-0.132	0.048	0.555	0.170
Pyruvic acid	0.102	-0.503	0.389	0.528	-0.215	1	-0.012	0.491	-0.656	-0.661
Acetaldehyde	-0.180	-0.341	-0.058	-0.131	-0.132	-0.012	1	0.327	-0.382	-0.406
L*	0.203	-0.210	0.504	0.523	0.048	0.491	0.327	1	-0.534	-0.618
a*	0.441	0.657	-0.348	-0.175	0.555	-0.656	-0.382	-0.534	1	0.392
b*	-0.297	0.435	-0.500	-0.492	0.170	-0.661	-0.406	-0.618	0.392	1

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References

Ansell, R., Granath, K., Hohmann, S., Thevelein, J.M., & Adler, L. (1997). The two isoenzymes for yeast NAD+-dependent glycerol 3-phosphate dehydrogenase encoded by GPD1 and GPD2 have distinct roles in osmoadaptation and redox regulation. EMBO Journal, 16, 2179–2187.

Ayala, F., Echavarri, J. F., & Negueruela, A. I. (1997). A new simplified method for measuring the color of wines. I. Red and rose wines. American Journal of Enology and Viticulture, 48(3), Article 357.

Bastard, A., Coelho, C., Briandet, R., Canette, A., Gougeon, R., Alexandre, H., Guzzo, J., & Weidmann, S. (2016). Effect of biofilm formation by Oenococcus oeni on malolactic fermentation and the release of aromatic compounds in wine. Frontiers in Food Microbiology, 7, Article 613.

Belda, I., Ruiz, J., Beisert, B., Navascués, E., Marquina, D., Calderón, F., Rauhut, D., Benito, S., & Santos, A. (2017). Influence of Torulaspora delbrueckii in varietal thiol (3-SH and 4-MSP) release in wine sequential fermentations. International Journal of Food Microbiology, 257, 183–191.

Berg, H. W., Filipello, F., Hinreiner, E. & Webb, A. D. (1955). Evaluation of thresholds and minimum difference concentrations for various constituents of wines. Water solutions of pure substances. Food Technology, 9, 23–26.

Bojsen, R. K., Andersen, K. S., & Regenberg, B. (2012). Saccharomyces cerevisiae-a model to uncover molecular mechanisms for yeast biofilm biology. FEMS Immunology and Medical Microbiology, 65(2), 169–182.

Caridi, A., Sidari, R., Kraková, L., Kuchta, T., & Pangallo, D. (2015). Assessment of color adsorption by yeast using Grape Skin Agar and impact on red wine color. OENO One, 49(3), 195–203.

Cartus, A. T., Lachenmeier, D. W., Guth, S., Roth, A., Baum, M., Diel, P., Eisenbrand, G., Engeli, B., Hellwig, M., Humpf, H-U., Joost, H-G., Kulling, S.E., Lampen, A., Marko, D., Steinberg, P., Wätjen, W., Hengstler, J.G., & Mally, A. (2023). Acetaldehyde as a Food Flavoring Substance: Aspects of Risk Assessment. Molecular Nutrition & Food Research, Article 2200661.

Comitini, F., Agarbati, A., Canonico, L., & Ciani, M. (2021). yeast interactions and molecular mechanisms in wine fermentation: A comprehensive review. International Journal of Molecular Science, 22, Article 7754.

Desai, J. V., Bruno, V. M., Ganguly, S., Stamper, R. J., Mitchell, K. F., Solis, N., Hill, E. M., Xu, W., Filler, S. G., Andes, D. R., Fanning, S., Lanni, F., & Mitchell, A. P. (2013). Regulatory role of glycerol in Candida albicans biofilm formation. MBio, 4, Article e00637–00612.

Donlan, R. M., & Costerton, J. W. (2002). Biofilms: Survival mechanisms of clinically relevant microorganisms. Clinical Microbiology Reviews, 15(2), 167–193.

Echeverrigaray, S., Scariot, F. J., Menegotto, M., & Delamare, A. P. L. (2020). Anthocyanin adsorption by Saccharomyces cerevisiae during wine fermentation is associated to the loss of yeast cell wall/membrane integrity. International Journal of Food Microbiology, 314, Article 108383.

Englezos, V., Pollon, M., Rantsiou, K., Ortiz-Julien, A., Botto, R., Río Segade, S., Giacosa, S., Rolle, L., & Cocolin, L. (2019). Saccharomyces cerevisiae-Starmerella bacillaris strains interaction modulates chemical and volatile profile in red wine mixed fermentations. Food Research International, 122, 392–401.

Escot, S., Feuillat, M., Dulau, L., & Charpentier, C. (2001). Release of polysaccharides by yeast and the influence of polysaccharides on colour stability and wine astringency. Australian Journal of Grape Wine Research, 7, 153–159.

Escribano-Viana, R., Portu, J., Garijo, P., López, R., Santamaría, P., & López-Alfaro, I. (2019). Effect of the sequential inoculation of non-Saccharomyces/Saccharomyces on the anthocyans and stilbenes composition of Tempranillo wines. Frontiers in Food Microbiology, 10, Article 773.

European Parliament and Council (2008) Regulation (EC)No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives67/548/EEC and 1999/45/EC, and amending Regulation (EC) No1907/2006. 2008

Evers, M. S., Roullier-Gall, C., Morge, C., Sparrow, C., Gobert, A., & Alexandre, H. (2021). Vitamins in wine: Which, what for, and how much?. Comprehensive Reviews In Food Science and Food Safety, 20(3), 2991–3035.

Ge, Q., Guo, C.; Yan, Y. Sun, X., Ma, T., Zhang, J., Li, C., Gou, C., Yue, T., & Yuan Y. (2022). Contribution of non-Saccharomyces yeasts to aroma-active compound production, phenolic composition and sensory profile in Chinese Vidal icewine. Food Bioscience, 46, Article 101152.

Guilhen, C., Charbonnel, N., Parisot, N., Gueguen, N., Iltis, A., Forestier, C., & Balestrino, D. (2016). Transcriptional profiling of Klebsiella pneumoniae defines signatures for planktonic, sessile and biofilm-dispersed cells. BMC Genomics, 17, Article 237.

Ivit, N. N., Longo, R., & Kemp, B. (2020). The effect of non-Saccharomyces and Saccharomyces non-cerevisiae yeasts on ethanol and glycerol levels in wine. Fermentation, 6, Article 77.

Jolly, N. P., Varela, C., & Pretorius, I. S. (2014). Not your ordinary yeast: Non-Saccharomyces yeasts in wine production uncovered. FEMS Yeast Research, 14, 215–237.

Jagatić Korenika, A. M., Tomaz, I., Preiner, D., Plichta, V., & Jeromel, A. (2021). Impact of commercial yeasts on phenolic profile of Plavac Mali wines from Croatia. Fermentation, 7, Article 92.

Lemos Junior, W. J. F., de Oliveira, V. S., Guerra, A. F., Giacomini, A., & Corich, V. (2021). From the vineyard to the cellar: new insights of Starmerella bacillaris (synonym Candida zemplinina) technological properties and genomic perspective. Applied Microbiology and Biotechnology, 105(2), 493-501.

Lisov, N., Petrovic, A., Čakar, U., Jadranin, M., Tešević, V., & Bukarica-Gojković, L. (2020). Extraction kinetic of some phenolic compounds during Cabernet Sauvignon alcoholic fermentation and antioxidant properties of derived wines. Macedonian Journal of Chemistry and Chemical Engineering, 39, 185-196.

Liu, S. Q., & Pilone, G. J. (2000). An overview of formation and roles of acetaldehyde in winemaking with emphasis on microbiological implications. International Journal of Food Science and Technology, 35, 49–61.

Madžgalj, V., Petrović, A., Čakar, U., Maraš, V., Sofrenić, I., & Tešević, V. (2023). The influence of different enzymatic preparations and skin contact time on aromatic profile of wines produced from autochthonous grape varieties Krstač and Žižak. Journal of the Serbian Chemical Society, 88, 11–23.

Magyar, I., Nyitrai-Sárdy, D., Leskó, A., Pomázi, A., & Kállay, M. (2014). Anaerobic organic acid metabolism of Candida zemplinina in comparison with Saccharomyces wine yeasts. International Journal of Food Microbiology, 178, 1–6.

Magyar, I., & Tóth, T. (2011). Comparative evaluation of some oenological properties in wine strains of Candida stellata, Candida zemplinina, Saccharomyces uvarum and Saccharomyces cerevisiae. Food Microbiology, 28, 94–100.

Mangani, S., Buscioni, G., Collina, L., Bocci, E., & Vincenzini, M. (2011). Effects of microbial populations on anthocyanin profile of Sangiovese wines produced in Tuscany, Italy. American Journal of Enology and Viticulture, 62, 487–494.

Manzanares, P., Rojas, V., Genovés, S., & Vallés, S. (2000). A preliminar search for anthocyanin-ß-D-glucosidase activity in non-Saccharomyces wine yeasts. International Journal of Food Science and Technology, 35, 95–103.

Martinez, J. A., Melgosa, M., Perez, M. M., Hita, E., & Negueruela, A. I. (2001). Visual and instrumental color evaluation in red wines. Food Science and Technology International, 7, 439–444

Medina, K., Boido, E., Dellacassa, E., & Carrau, F. (2005). Yeast interactions with anthocyanins during red wine fermentation. American Journal of Enology and Viticulture, 56, 104–109.

Morata, A., Gómez-Cordovés, M. C., Colomo, B., & Suárez, J. A. (2003). Pyruvic acid and acetaldehyde production by different strains of Saccharomyces cerevisiae: relationship with Vitisin A and B formation in red wines. Journal of Agricultural and Food Chemistry, 51(25), 7402–7409.

Morata, A., Loira, I., Heras, J. M., Callejo, M. J., Tesfaye, W., Gonzalez, C., & Suarez-Lepe, J. A. (2016). Yeast influence on the formation of stable pigments in red winemaking. Food Chemistry, 197(Pt A), 686–691.

Morata, A., Benito, S., Loira, L., Palomero, E, González, M. C., & Suárez-Lepe, J. A. (2012). Formation of pyranoanthocyanins by Schizosaccharomyces pombe during the fermentation of red must. International Journal of Food Microbiology, 159, 47–53.

Morata, A., Loira, I., Vejarano, R., Bañuelos, M. A., Sanz, P. D., Otero, L., & Suárez-Lepe, J. A. (2015). Grape processing by high hydrostatic pressure: effect on microbial populations, phenol extraction and wine quality. Food and Bioprocess Technology, 8, 277–286.

Nadai, C., Giacomini, A., & Corich, V. (2021). The addition of wine yeast Starmerella bacillaris to grape skin surface influences must fermentation and glycerol production. OENO One, 55, 47–55.

Nisiotou, A., Sgouros, G., Mallouchos, A., Nisiotis, C.S., Michaelidis, C., Tassou, C., & Banilas, G. (2018). The use of indigenous Saccharomyces cerevisiae and Starmerella bacillaris strains as a tool to create chemical complexity in local wines. Food Research International, 111, 498–508

Organisation International de la Vigne et du Vin (OIV). (2023). Compendium of international methods of wine and must analysis.

Padilla, B., Gil, J. V., & Manzanares, P. (2016). Past and future of non-Saccharomyces yeasts: From spoilage microorganisms to biotechnological tools for improving wine aroma complexity. Frontiers in Food Microbiology, 7, 1–20.

Pannella, G., Lombardi, S. J., Coppola, F., Vergalito, F., Iorizzo, M., Succi, M., Tremonte, P., Iannini, C., Sorrentino, E., & Coppola, R. (2020). Effect of biofilm formation by Lactobacillus plantarum on the malolactic fermentation in model wine. Foods, 9(6), Article, 797.

Perpetuini, G., Rossetti, A. P., Tittarelli, F., Battistelli, N., Arfelli, G., Suzzi, G., & Tofalo, R. (2021). Promoting Candida zemplinina adhesion on oak chips: A strategy to enhance esters and glycerol content of Montepulciano d'Abruzzo organic wines. Food Research International, 150, Article 110772.

Perpetuini, G., Tittarelli, F., Perla, C., & Tofalo, R. (2022). Influence of different aggregation states on volatile organic compounds released by dairy Kluyveromyces marxianus strains. Foods, 11(18), Article 2910.

Perpetuini, G., Rossetti, A. P., Battistelli, N., Zulli, C., Piva, A., Arfelli, G., Corsetti, A., & Tofalo, R. (2023). Contribution of Starmerella bacillaris and oak chips to Trebbiano d'Abruzzo wine volatile and sensory diversity. Foods, 12(5), Article 1102.

Pourcelot, E., Conacher, C., Marlin, T., Bauer, F., Galeote, V., & Nidelet, T. (2023). Comparing the hierarchy of inter- and intra-species interactions with population dynamics of wine yeast cocultures. FEMS Yeast Research, 23, Article foad039.

Rossouw, D., Meiring, S. P., & Bauer, F. F. (2018). Modifying Saccharomyces cerevisiae adhesion properties regulates yeast ecosystem dynamics. mSphere, 3(5), Article e00383-18.

Rossouw, D., Bagheri, B., Setati, M. E., & Bauer, F. F. (2015). Co-flocculation of yeast species, a new mechanism to govern population dynamics in microbial ecosystems. PloS one, 10(8), Article e0136249.

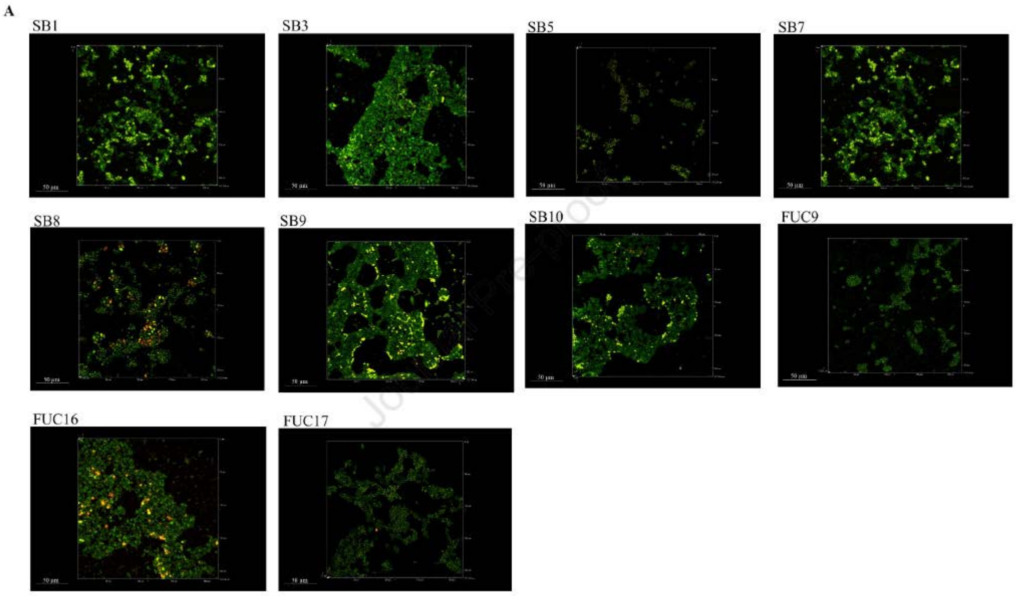
Russo, P., Tufariello, M., Renna, R., Tristezza, M., Taurino, M., Palombi, L., Capozzi, V., Rizzello, C. G., & Grieco, F. (2020). New insights into the oenological significance of Candida zemplinina: Impact of selected autochthonous strains on the volatile profile of Apulian wines. Microorganisms, 8, Article 628

Suzzi, G., Arfelli, G., Schirone, M., Corsetti, M., Perpetuini, G., & Tofalo, R. (2012). Effect of grape indigenous Saccharomyces cerevisiae strains on Montepulciano d'Abruzzo red wine quality. Food Research International, 46, 22–29.

Tofalo, R., Patrignani, F., Lanciotti, R., Perpetuini, G., Schirone, M., Di Gianvito, P., Pizzoni, D., Arfelli, G., & Suzzi, G. (2016). Aroma profile of Montepulciano d'Abruzzo wine fermented by single and co-culture starters of autochthonous Saccharomyces and non-Saccharomyces yeasts. Frontiers in Food Microbiology, 7, Article 610.

Tofalo, R., Suzzi, G., & Perpetuini, G. (2021). Discovering the influence of microorganisms on wine color. Frontiers in Food Microbiology, 12, Article 790935.

Tofalo, R., Schirone, M., Torriani, S., Rantsiou, K., Cocolin, L., Perpetuini, G., & Suzzi, G. (2012). Diversity of Candida zemplinina strains from grapes and Italian wines. Food Microbiology, 29, 18–26.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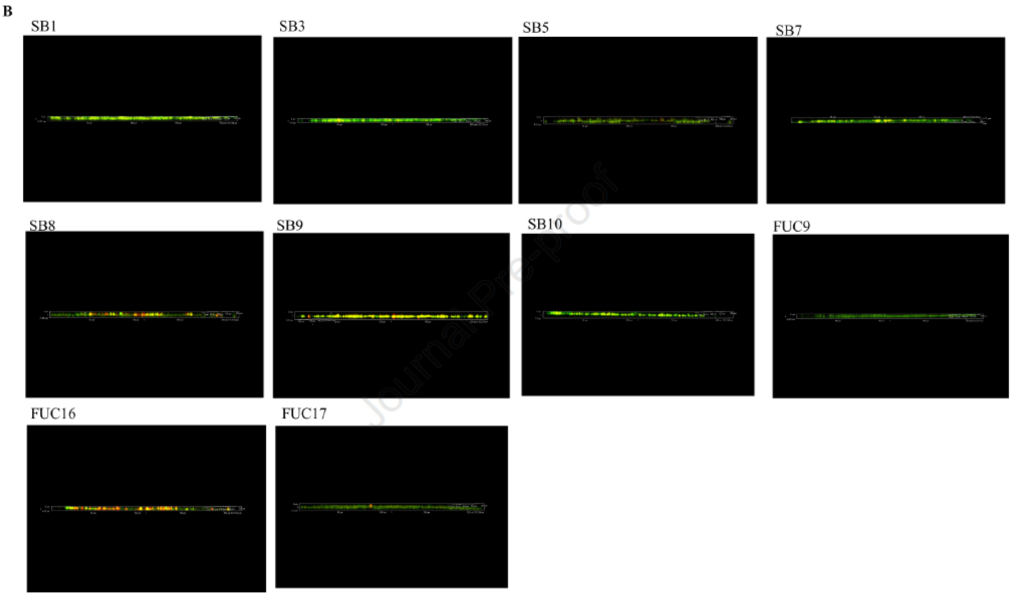

Figure 1

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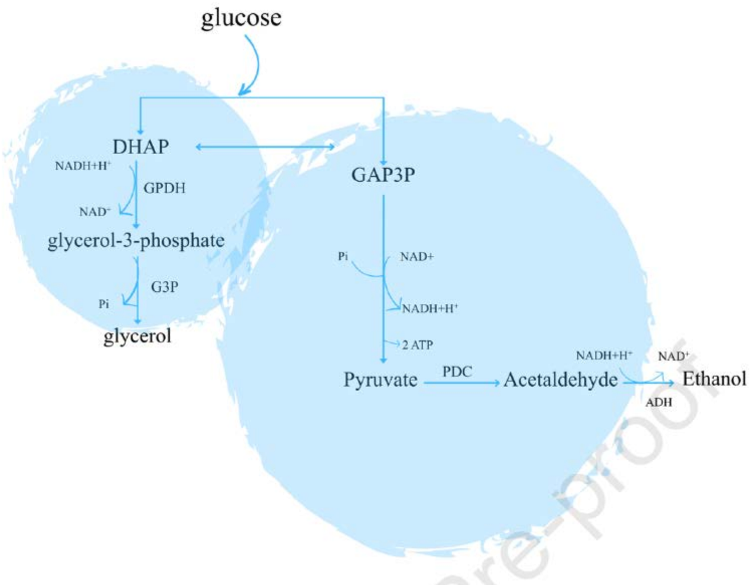

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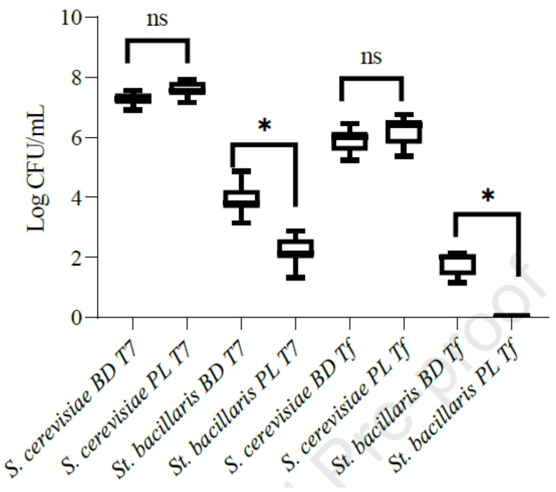
Figure 2.



Log CFU/mL
10
8
6
4
2
0
ns
*
ns
*
S. cerevisiae BD T7
S. cerevisiae PL T7
St. bacillaris BD T7
St. bacillaris PL T7
S. cerevisiae BD Tf
S. cerevisiae PL Tf
St. bacillaris BD Tf
St. bacillaris PL Tf

Figure 3.

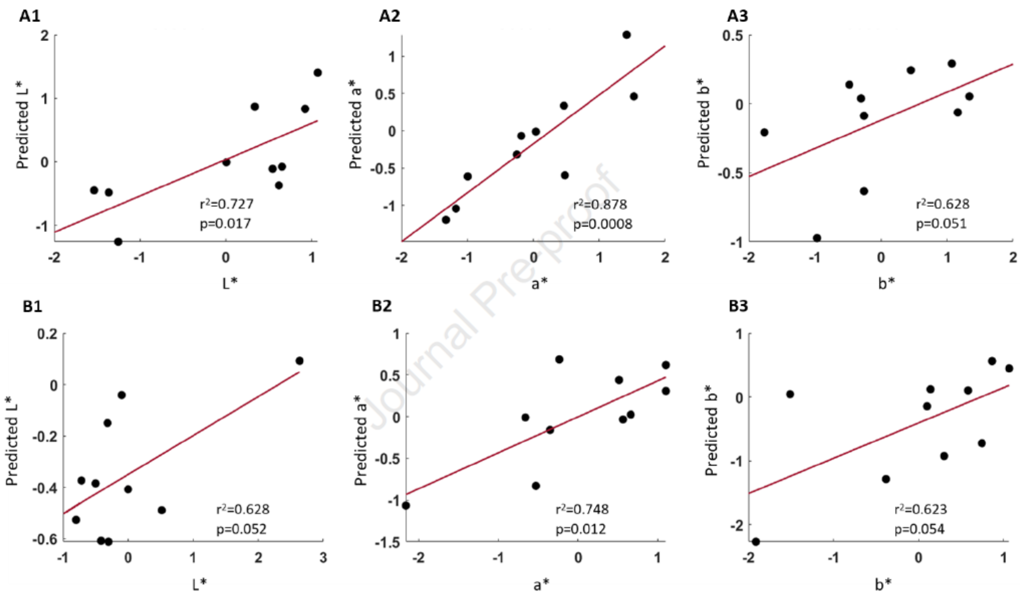


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Figure 4.